Restoration of Nitrogen Cycling in a Degraded Shrubland Ecosystem

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Department of Biochemistry
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Abstract

Effects on native ecosystems caused by human disturbance or non-native species invasions can persist far longer than the initial activity, particularly if soil properties such as nutrients are altered. Soil legacy effects present a complex challenge for restoration, because both plants and microbes play important roles in soil biogeochemical cycling. We examined whether nitrogen cycling could be restored by removing non-native vegetation and inoculating degraded sites with native soil microbial communities. These strategies were applied in degraded Florida shrublands that were mildly disturbed or entirely converted to pasture, or undisturbed native control sites. We measured inorganic nitrogen pools, gross rates of nitrogen mineralization, and gross rates of nitrogen consumption 2 years after the treatments were implemented. Gross rates were quantified using the pool dilution technique. We found that gross N mineralization rates, gross N consumption rates, and N pool sizes increased with increasing disturbance. In disturbed sites, non-native vegetation removal and microbial addition individually decreased gross rates, and effectively restored native N conditions. In pastures, the combination of both treatments was most effective, but resulted in an elevated inorganic nitrate pool. Disturbance compounded by non-native invasion increases soil legacy effects by altering soil nutrient dynamics, but responds proportionally to a restoration scheme to reapproximate native N cycling rates.

Key Words: Restoration ecology, ^{15}N pool dilution, EA-IRMS, Florida xeric shrubland, sandy soil restoration, Nitrogen Cycling, soil biogeochemistry, stable isotope biogeochemistry

Introduction

Anthropogenic land transformation alters vegetation, soil characteristics, and biogeochemical nutrient cycling that persists far beyond human tenure on the land. Such legacies are particularly common when non-native plant species invade ecosystems (Elgersma, *et al.* 2011) and are well known to continue after removal of the non-natives (Foster *et al.,* 2003). Soil and biogeochemical legacy effects from invasions complicate subsequent ecosystem restoration efforts and, if unmitigated, can maintain a new landscape equilibrium (Wardle & Peltzer, 2017). It remains critical to biodiversity and future land management to develop strategies to successfully overcome legacy effects (Dobson *et al.,* 1997).

In practice, restoration strategies require differing methods and approaches that depend on the environmental history and the overall goals of a project, but they can benefit from simultaneously applying a variety of techniques as well as a broad understanding beyond quantitative outcomes towards ecosystem resilience and variability (Hilderbrand *et al.,* 2005). To address soil and biogeochemical legacies requires restoration targeting both aboveground and belowground ecosystems (Wardle *et al.,* 2004). For example, non-native plant invasions can significantly alter soil microbial communities and nitrogen (N) cycling compared to uninvaded sites (Kourtev *et al.* 2002). Shifts in the N cycle can be generated directly by non-native plants via physiological characteristics that differ from natives, but these effects can also be caused indirectly by how the non-natives affect the soil microorganisms that control transformations in the N cycle. Our understanding of legacy effects caused by disturbance and invasion is thus also tied to soil biogeochemistry and microbial response, which is broadly known but not specifically understood.

Alterations to the N cycle are particularly important because N is an essential element for all life. In undisturbed ecosystems, N is usually a limiting nutrient and its abundance constrains

the productivity, composition, dynamics and diversity of both plants and soil microorganisms (Vitousek et. al., 2002; Lange *et al.,* 2014). To increase productivity and grow crops or create an environment suitable for pasture, fertilizer is added to increase soil N levels (Blumenthal, 2005). Invasions of non-native plants often increase soil N as well, through a combination of fast growth, shifted tissue quality, and organic matter accumulation (Ehrenfeld, 2003).

If unbalanced or oversaturated, especially in nutrient poor systems, N can alter plant composition and diversity by changing plant metabolism and favoring non-native species. For example, non-native plants that maintain high N mineralization rates due to low tissue C:N ratios and can outperform natives in artificially high resource environments (Milchunas and Lauenroth, 1995; Blumenthal, 2005). In this way, non-native invasions that change N can create large and lasting effects in the ecosystem, creating an environment friendly to continued invasion for decades or longer as has been recorded in areas ranging from hardwood forest to semiarid grassland (Evans and Belnap, 1999; Goodale and Aber, 2001; Hawkes *et al.,* 2005, 2006). Land-use history may then become the best predictor of N cycling rates, conditioning land response to future N inputs as a legacy effect (Goodale and Aber, 2001; Foster *et al.,* 2003).

We argue that it is important to address soil N and microbial N controllers directly in restoration, rather than simply removing non-natives and returning native plants, because the N cycle is integral for regulating normal ecosystem function or perpetuating altered ecosystem characteristics. Here, we tested this idea in disturbed shrubland at the Archbold Biological Station in south central Florida. We evaluated how restoration treatments aimed at removing non-native vegetation vs. inoculating native soil microorganisms mitigated soil N legacies. Specifically, we measured changes in soil N pools and gross rates of N mineralization and consumption. We applied the treatments in sites that differed in their degree of disturbance and

invasion to gauge how treatment success depended on the magnitude of the change needed. Based on previous work in which N pools increased with the degree of disturbance (Hamman and Hawkes, 2013), we expected that N cycling rates would similarly increase with degree of disturbance compared to native sites. We also expected that the combination of non-native vegetation removal and microbial amendments would be the most beneficial for restoring N cycling, and that these would have the largest benefit in the least disturbed ecosystems.

Materials and Methods

Site Description

Sites with three different disturbance levels were considered at the Archbold Biological Station located on the Lake Wales Ridge in Venus, Florida: undisturbed scrub (native), disturbed scrub, and pasture sites. Native scrub sites are a xeric matrix of dominant, pyrogenic *Ceratiola ericoides* (Florida rosemary shrubs) and scrubby flatwoods with open sand gaps. These open sand gaps harbor endemic and endangered herbaceous plants (Menges et al. 2008). Native soils also contain biological crusts that aggregate in the top 1 cm of sand and are made up of algae, cyanobacteria, fungi, bacteria, and associated extracellular polysaccharides. These organisms form a fragile but cohesive mat and are known to affect seed germination and N cycling (Hawkes & Fletchner 2002; Hawkes 2003, 2004). There are no non-native grasses in native scrub sites (Hamman & Hawkes 2013). Disturbed scrub sites were roller-chopped in 1995 and invaded by *Melinis repens* (Willd.) Zizka (Natal grass), an annual or short-lived perennial, obligate-seeding, warm-season grass native to South Africa. The pasture sites represent a near-complete conversion of native scrub and are now dominated by non-native *Paspalum notatum* Flueggé (Bahia grass), a perennial, rhizomatous, warm-season grass from South America. Pasture sites were roller-chopped, burned, fertilized, and seeded in the late 1970's and grazed continuously

through 2004. Undisturbed native scrub soils are sandy, well drained, and nutrient poor; both disturbed and pasture sites have elevated soil N (Hamman and Hawkes 2013).

Field Restoration Treatments

We used two restoration treatments with the aim of directly affecting N cycling: herbicide to remove aboveground non-native vegetation (except for the native sites) and native microbial community additions. Five sites from each vegetation type were selected in May 2006 with treatments applied in 3x3m plots. Disturbed and pasture sites were selected in areas with the highest density of non-native grasses. In August and December 2006, 2% glyphosate (Roundup®, Monsanto, St. Louis, MO, U.S.A.) was applied to half of the plot which had a 50m radius. In December 2006 and May 2007, soil microbes were added to only control subplots in native sites and control as well as removal subplots in in disturbed and pasture sites. The top 2 cm of soil was collected from fire lanes located at the edges of native sites, representing the biological soil crust layer that plays an important role in the N cycle of these xeric, sandy soils (Hawkes 2003). The soil inoculum was homogenized through mixing and added as a 1-cm depth layer across treatment plots. The full experimental design is shown in Figure 1: pasture and disturbed sites underwent grass removal, microbial addition, and grass removal with microbial addition, while native sites were treated with microbial addition only.

Field Sampling

Pool dilutions were used to measure gross rates of N cycling 30 months after treatments were implemented. In each replicate plot, PVC cores (5 x 15 cm) for pool dilutions were emplaced three months prior to sampling to limit disturbance effects. In June 2009, 1 mL of 99 at% $(^{15}NH_4)_2SO_4$ was injected into each soil core using a 3-mm diameter steel syringe, spread across five injection points per core, resulting in a total of 26 μg ^{15}N added per core. Cores were

collected within 5 min and 24 hr after injection. Soils were kept on ice for immediate transport back to the lab.

Measuring N Pool Size

Soils were homogenized and inorganic NH_4^+ and NO_3^- were determined by extracting in 2:1 2M KCl to soil. Colorimetric microplate analysis measured ammonium and nitrate content in each sample, quantified at the University of Texas at Austin ICMB core facility. To determine ammonium content, the indophenol-blue method for low concentration samples was used, combining 40 µl sample with 80 µl salicylate solution and 80 µl bleach solution, developed for 1 hour and read at 667 nm (Verdouw *et al.*, 1977, Weatherburn 1967). Nitrate content was quantified by adding 400 mg vanadium (III) chloride to 50 ml 1M HCl and mixed with 200 mg of sulfanilamide, 10 mg N-(1-naphthyl) ethylenediamine dihydrochloride, and 100 ml 1M HCl. Replicates of 5:4 sample to reagent (85 µl sample: 68 µl VCl_3 solution) were plated and developed for 16 hours at room temperature. Absorbance was measured at 540 nm (Doane & Horwath, 2003, Mulvaney, 1996).

Measuring Mineralization/Consumption Rates

Pool dilutions were performed to indicate changes in microbial activity in response to changes in substrate availability (Murphy et al., 2003). The technique uses ^{15}N as a tracer to indicate the gross rates of N transformation. In combination with the soil core method, which avoids problematic soil mixing, pool dilutions allow for an accurate measurement of N mineralization, nitrification, and immobilization (Davidson et al., 1991). To summarize the method, mineralization is measured by adding $^{15}NH_4$ to an NH_4^+ sample pool to determine the rate at which ^{15}N enrichment declines over time as the more dominant form, ^{14}N, is mineralized to $^{14}NH_4^+$ by microorganisms in the soil. Immobilization is determined by measuring the decline of

^{15}N due to consumption of NH_4^+ and NO_3^- by soil microorganisms; however, loss of NH_4^+ can also occur through other processes. For the purposes of this paper, consumption rates include all immobilization (i.e., microbial assimilation), autotrophic nitrification, volatilization, and all other possible NH_4^+ transformations (Davidson *et al.* 1991).

To assess ^{15}N content, both NH_4^+ and NO_3^- extracts were diffused onto acidified paper disks following (Brooks *et al.*, 1989, Herman *et al.*, 1995) and analyzed for ^{15}N on a Delta V isotope ratio mass spectrometer (IRMS) with elemental analyzer (EA) and Conflo IV instrument. Standards bracketed every ten samples to account for instrument drift and a standard curve was run at the end of each set. A single standard curve was created from all standards in all runs to calculate the amount ^{15}N of each sample. Values were corrected for instrument blanks and method blanks.

Calculations

Gross mineralization and consumption rates were calculated using time results from EA-IRMS analysis at time 0 h and time 24 h. Calculations were based on the Davidson *et al.* (2001) paper who adapted their equations based on Kirkham and Bartholomew (1954).

$$\boldsymbol{m = \frac{M_0 - M_1}{t} \times \frac{log(\frac{H_0 M_1}{H_1 M_0})}{log(\frac{M_0}{M_1})}}$$

$$\boldsymbol{c = \frac{M_0 - M_1}{t} \times \frac{log(\frac{H_0}{H_1})}{log(\frac{M_0}{M_1})}}$$

where $m \neq c$ and
M_0 = initial $^{14+15}N$ pool (μg N g^{-1} dry soil)
M_1 = post-incubation $^{14+15}N$ pool (μg N g^{-1} dry soil)
H_o = initial ^{15}N pool (μg N g^{-1} dry soil)
H_1 = post-incubation ^{15}N pool (μg N g^{-1} dry soil)
M = mineralization rate (μg N g^{-1} dry soil d^{-1})
c = consumption rate (μg N g^{-1} dry soil d^{-1})
t = time (1 d for the present study)

Statistical Analysis

We assessed how gross N mineralization rates, gross N consumption rates, NH_4^+ pools, and NO_3^- pools were affected by vegetation type (native, disturbed, pasture), non-native removal, and microbial inoculation using a linear mixed model (afex package; lmer, Kenward-Roger method). Vegetation type and each treatment were treated as fixed effects, plot was treated as a random effect, and treatments were nested within vegetation type to account for the hierarchical design. The overall design was unbalanced because native scrub did not include vegetation removal, so direct comparisons between native scrub and treatment combination plots could not occur. To examine the remaining possible interaction effects, Tukey contrasts were used to make multiple comparisons of means (lsmeans package, Satterthwaite approximations of degrees of freedom for t tests). All analyses were completed in R (version 3.3.1). All means are reported with ± 1 SE.

Results

Comparison of Vegetation Types in Untreated Control Plots

Rates of N cycling increased as disturbance increased across vegetation types, with up to 5-fold higher gross N mineralization (Figure 2a) and 90-fold higher gross N consumption rates in untreated pasture compared to native scrub (Table 1, Figure 2b). Increased mineralization rates in pasture were also reflected in the NH_4^+ pool (Figure 3), which was 2.66 fold larger than in native scrub. In contrast, both untreated disturbed sites and native scrub had low rates of gross N mineralization and consumption, as well as low NH_4^+ and NO_3^- pools. Although they did not differ significantly, untreated disturbed sites had nearly double gross N mineralization and 20 times gross N consumption rates on average compared to native scrub (Table 1, Figure 2a). NO_3^- pools were similar across all untreated sites (Table 2, Figure 3).

Non-native Vegetation Removal

Removal of non-native grasses reduced gross N mineralization and consumption rates in pasture sites by 55% and 71%, respectively, compared to untreated plots (Table 1, Figure 2a, 2b). The NH_4^+ pool size was also reduced by 63%, whereas NO_3^- pool size rose by 3.69-fold (Table 2, Figure 3). In disturbed sites, gross rates of N mineralization after non-native grass removal were 49% lower than the untreated plots and consumption rates were 58% lower on average, though N pool sizes followed the same trends as for pasture, decreasing by 12% and increasing by 2.4-fold for NH_4^+ and NO_3^- pool sizes respectively.

When compared to native scrub, pasture vegetation removal, though lower than before treatment, remained greater in all respects. Pasture maintained a 2.5 higher gross rate of N mineralization, 34-fold higher consumption rate, 1.67-fold greater NH_4^+pool size, and 9.24-fold higher nitrate pool size but was not statistically different from native scrub controls. Gross N mineralization (6% lower) and consumption rates (9.3 fold higher), and ammonium pool size (20% lower) in disturbed sites was not only no longer statistically different from native scrub when non-native vegetation was removed, but also below control ammonium pool size and mineralization rates (Figure 3, Table 2).

Microbial Addition

Microbial addition treatments follow a similar pattern to vegetation removal, further lowering mineralization rates in pastures . Gross N mineralization in pastures were significantly reduced by 52% and consumption rates reduced by 50% compared to in-site untreated control plots after microbial addition, but remained 2.6- and 47-fold greater than in native scrub sites, respectively (Table 1, Figure 2). The NH_4^+ pool was similarly decreased by 63% but the NO_3^- pool was increased by 36% with microbial addition (Figure 3). In disturbed sites, neither gross rate nor the nitrate pool was altered significantly with microbial addition compared to untreated control plots,

although NH_4^+ and NO_3^- pools increased by 8% and 31% respectively (Figure 2, 3, Table 2). Native scrub consumption after microbial addition was 6 fold greater than untreated native scrub sites.

Compared to vegetation removal, microbial addition was less effective at lowering gross N consumption rates and more effective lowering mineralization rates. While microbial addition stabilized NO_3^- pools in pastures, it otherwise had similar effects as vegetation removal on the ammonium pool (Figure 2, 3).

Combined Vegetation Removal and Microbial Addition

The combination of native soil microbial inoculum and vegetation removal significantly lowered gross N mineralization and consumption rates in pasture sites by 70% and 86% compared to untreated controls (Figure 2, posthoc tests). The combination of treatments additionally lowered NH_4^+ in pasture sites by 41% and raised NO_3^- to 4.66-fold above untreated pasture control plots. Although gross rates remained 65% and 12.8-fold elevated above the native scrub target, they were not significantly different (Table 1). In disturbed scrub, the addition of native microbial inoculum after vegetation removal reduced gross N mineralization rates by 53% compared to untreated control plots which were not considered different from native scrub sites. Gross N consumption rates in disturbed sites were also reduced by 20% compared to untreated control plots, but remained 11-fold higher than in native sites. In disturbed scrub, NH_4^+ was 42% lower than either untreated control plots or native scrub, but NO_3^- increased by 2.1 and 2.6, respectively (Figure 3).

Compared to vegetation removal or microbial addition treatments applied individually, their combined application in pastures further lowered rates of gross N mineralization by 42-53% and further lowered rates of gross N consumption by 63-73%. NH_4^+ pool sizes were unchanged

between the single and combined treatments, but NO_3^- was elevated in pastures on par with vegetation removal alone (Table 2). In contrast, for disturbed sites gross N mineralization rates were either unchanged with crust addition or within 20% of control sites while consumption rates with the treatment combination remained within 14-20% of single treatments.

Discussion

We found that invasion, and especially invasion combined with substantial disturbance in pastures, created soil legacy effects in the form of increased N cycling and N pool sizes when compared to undisturbed native scrub. However, those legacies were considerably reduced or entirely removed after 30 months of restoration treatments. These restoration effects are likely to persevere based on the return of gross N cycling rates to or near to normal. Thus, we demonstrate that near to full restoration of biogeochemical cycles can occur when both aboveground and belowground legacies are directly addressed.

The strength of the N legacy effect varied with how much the original landscape was altered by invasion and disturbance. This is consistent with previous measurements of N pools at this site made at 17 months post-treatment (Hamman and Hawkes 2013) that showed elevated levels within disturbed and pasture areas, suggesting that the legacy would persist without intervention. Other studies have found similar impacts on N cycling from non-natives, which may be more likely in low-fertility systems such as Florida scrub (reviewed in Corbin and D'Antonio 2004). Legacy persistence may be due to new ecological niches created by increased N that favor continued dominance by non-native species (Penuelas *et al.*, 2010). Conversely, effects of non-native species with lesser impacts on N should dissipate more rapidly once removed, consistent with disturbed site results in this study.

Restoration treatment effectiveness depended on vegetation type and N legacy. The combination of vegetation removal and microbial addition was the most effective treatment in pastures, reducing gross N mineralization and consumption to near rates found in native scrub. This contrasts with disturbed sites, where vegetation removal alone was sufficient to meet N restoration goals. The combination may have been most effective for pastures because of the density of nonnative Bahia grass, large ammonium pools, and lack of native microbial communities in the highly transformed site. We know from previous work that the pastures have distinct fungal communities, and these are difficult to shift towards native scrub sites (Glinka and Hawkes 2014). Successful recolonization of degraded by native microbial communities may require dispersal or inoculation after nutrient legacies have been reduced. Moreover, biological crust recovery from natural disturbances in these shrubland ecosystems can take decades (Hawkes & Fletchner, 2001),

Although overall treatments were successful in reducing N legacies, vegetation removal, both alone and in combination with microbial addition, increased nitrate pool size in both disturbed and pasture sites to levels far above native scrub. Previous work by Hamman and Hawkes (2013) reported elevated TIN between sites, but this study shows that the nitrate pool disproportionately contributes to this increase. The vegetation removal treatments included aboveground litter removal, but resulted in large inputs of dead roots belowground, particularly in pastures. This could increase the nitrate pool by reducing plant uptake and providing more organic matter for nitrate retention in the sandy soils. Elevated nitrate levels may also be a more persistent legacy effect in pasture sites given that consumption decreases with vegetation removal and the addition of microbes with vegetation removal could not rescue this effect. Targeted microbial amendments or nitrification inhibitors might be useful to address this

problem. For example, when dicyandiamide (DCD) was applied to grazed pasture soils, it reduced annual average NO_3^- concentration by 42% and reduced NH_3^- oxidizing bacterial populations (Di & Cameron, 2005, 2011), but nitrification inhibitor effects on the microbial population as a whole remain unclear (Ruser & Schulz, 2015). However, to fully determine N movement into the nitrate pool requires estimates of both nitrification rates and microbial biomass N, which were not measured here.

Despite the success of microbial additions post-vegetation removal to restore pasture N cycling, it is not feasible to use whole soil as an inoculum for efforts on a larger scale. Instead, restoration efforts would require cultivation and inoculation of microbes. Successful cultivation requires that we know substantially more about the biology of these complex microbial communities. For example, cultivation of soil crusts can lead to rapid establishment in the field, but at the expense of diversity (Antoninka et al. 2017). Similarly, commercial techniques for other microbial applications are being developed, which are usually successful in monoculture agriculture (Timmusk *et al.*, 2017) but less effective in restoration (Emam, 2017; Palmer *et al.*, 2016; Middleton *et al.*, 2015; Leonard & Lyons, 2015). More recently, biological soil crust nurseries are being developed where crusts are inoculated by slurry, watered, and shaded to enhance production; in 4 months sufficient crust was grown to treat 6000 m2 of degraded soil (Velasco Ayuso et al. 2017). The success of microbial treatments in large-scale restoration hinges on the development of cultivation and application methods that do not require further degradation of native sites.

Gauging restoration success is complex. Here, we only focused on restoration of N cycling and only measured N cycling at one date. The amount of bioavailable N has been found to fluctuate based on drought and moisture conditions, increasing mineral N during drought

(Williams and Eldridge, 2011) which raises additional questions concerning N dynamics outside of summer season conditions. We also addressed only a small portion of the N cycle, despite other contributions that might be important. For example, in this system, N fixation is known to be important (Hawkes, 2003) and may have contributed to measured N pools. In addition, gross N mineralization rates in native scrub control plots were highly variable. In Florida xeric shrubland, plants less than 1m apart can experience a different microbial environment (Hawkes & Fletchner, 2001), which could directly affect N flux measurements (Evans & Johansen, 1999).Local flora recovery is also important for gauging and completing restoration efforts (Ruiz-Jaen & Aide, 2005), as is recovery of other community and ecosystem properties. In previous work, Hawkes and Hamman (2013) showed that plant recovery in this system was enhanced by both vegetation removal and crust addition, but the effects varied across years. Thus, the effects of restoration treatment must be measured over a longer time period and with a whole-ecosystem approach to understand long-term success.

Many have found that land use history is useful for identifying legacy effects that need to be addressed and that the level of land transformation can dictate the level of subsequent restoration success (Foster *et al.*, 2003; Hamman & Hawkes, 2003). As discussed in this paper, increasing involvement is needed depending on the level of disturbance as vegetation removal is sufficient in disturbed sites, but vegetation removal supplemented with native microbial addition is needed in pastures. Depending on the goal and the potential for an ecosystem to return to that target state, local methods may be useful to return diversity, vegetation, and ecological processes but it requires increased time and careful effort to establish and implement these drivers (Ruiz-Jaen & Aide, 2005; McKay *et al.*, 2005). In addition, success in one site may not translate to effective application in another even if two ecosystems have similar characteristics, and we must

acknowledge and plan for our limited predictive abilities in these areas (Hilderbrand *et al.,* 2005; Vidra & Shear, 2008). For example, while vegetation removal was overall effective at lowering N cycling rates, it had a greater impact for pasture consumption rates than mineralization rates, but was slightly more effective for all N dynamics measured in disturbed scrub. Natural ecosystem variability can complicate decision making when formulating an effective experimental design (Lange *et al.,* 2014) which blurs hard and fast goals, but we can also adapt to such realities and accept progress in new local equilibrium states. Other experiments seek to use this variability to their advantage, as Stefano *et al.* (2016) incorporated broader legal, ecosystem resilience and cost analysis factors into a Brazil savanna restoration projects to demonstrate the positive action resulting from the integrated and heterogenous nature of the project. While such analyses naturally avoid the most degraded areas that need the most help for cost purposes, they may encourage restoration where it was previously thought unfeasible and create a near biological resource for more site-specific methods to be applied in the future (McKay *et al.,* 2005, Vidra & Shear, 2008). Restoration benefits from an approach that discourages a generalized application of methods based purely on previous success, rather seeking a contextual but reasonable strategy that can still combine integral ecosystem characteristics with known impacts on the land.

Addressing soil legacies by both aboveground and belowground methods is important for promoting restoration and in the process better understand the links between ecosystem processes in nutrient poor environments. We found that N cycling changes associated with land use legacies could be effectively addressed, but the level of success depended on the degree of disturbance and invasion. By continuing to acknowledge the link and interplay between

aboveground and belowground processes, more informed restoration decisions can be made on the extent and efficacy of different strategies if land use legacy is known.

Conclusions

- Both aboveground vegetation removal in combination with belowground microbial addition methods can restore N cycling rates in highly transformed pasture sites, while vegetation removal alone is effective for disturbed areas. Yet legacy effects remain within nitrate pools.
- Disturbance level is an important indicator of legacy formation and subsequent restoration success.
- Clear restoration goals and specific gauges combined with site-specific knowledge are important to gauge new site equilibrium's.

Future Work

I diffused and prepared additional samples (~500/1700 total samples) at three other dates (December 2008, September 2009, January 2010) for both the nitrate and ammonium pools. The specific set analyzed in this thesis also has a suite of samples for parameters centering around the nitrate pool (nitrification). These samples can be run on the EA-IRMS to provide a more complete picture of change and seasonality over time within the N cycle. Especially with the extra data, the amount of ^{15}N in atom% excess can be modeled using FLUAZ courtesy of Mary *et al.* (1998) to acquire additional N cycling rates and the results added to the statistical model. Additional data concerning microbial crust composition and abundance as well as total microbial biomass N for each site and treatment are available or being processed for incorporation into a

future manuscript. This thesis will serve as a preliminary draft for a manuscript to be submitted to the journal *Restoration Ecology*.

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Biography
Elisa R. S. Friedmann was born in Albuquerque, New Mexico on June 30th, 1995. She enrolled in the Plan II Honors program at the University of Texas at Austin in 2013 and studied Biochemistry with a certificate from the Bridging Disciplines Program in Environment and Sustainability along with Plan II. In college, she founded the Longhorn Stream Team, a citizen science organization that teaches students how to paddle whitewater and monitor water quality on stretches otherwise hard to reach in Texas rivers. Ms. Friedmann received an Undergraduate Research Fellowship to support this project. She graduated December 2017 and will continue to work with Dr. Christine Hawkes and Dr. Daniel Breecker doing stable isotope biogeochemistry in the upcoming semester.

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Figure 1. The experimental design with three vegetation types and two treatments: vegetation removal and subsequent microbial addition. Note that the native scrub plots did not undergo vegetation removal. Control plots indicate that neither vegetation removal nor microbial addition was applied to the plot.

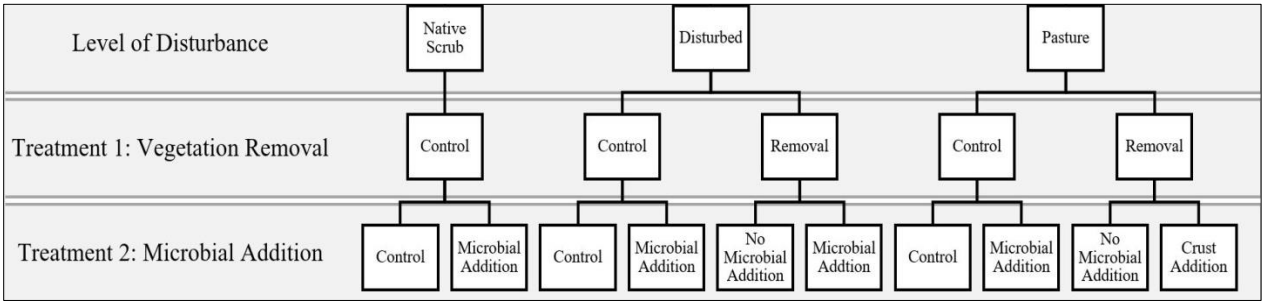


Table 1. Reported linear mixed model degrees of freedom, F ratios, and significance test for the effects of vegetation type, vegetation removal, microbial addition, and interactions between fixed factors on mineralization and consumption rates.

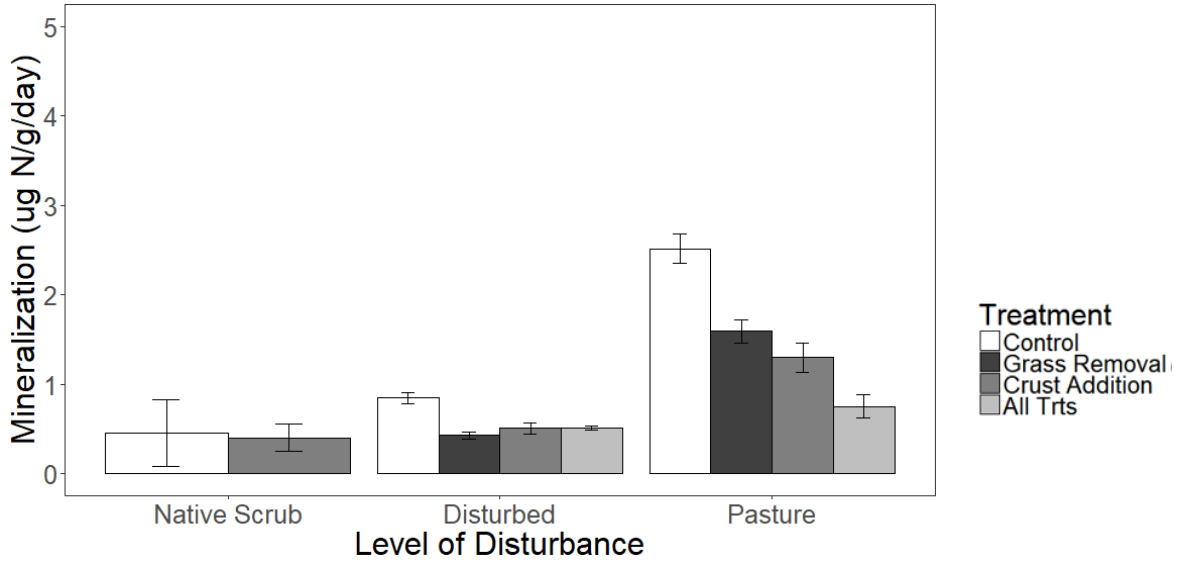
	Mineralization			Consumption		
Between Subjects	**df**	**F**	**p value**	**df**	**F**	**p value**
VegType	18.83	11.78	**<0.001**	18.98	10.12	**0.001**
Crust	17.73	7.15	**0.020**	19.04	1.83	0.190
Herb	17.52	4.61	**0.050**	17.35	7.18	**0.020**
Veg:Herb	17.52	1.51	0.240	17.35	3.54	0.080
Veg:Crust	17.18	8.07	**0.003**	18.26	3.59	**0.050**
Veg:Herb:Crust	16.94	0.09	0.770	17.65	0.54	0.470

Table 2. Reported linear mixed model degrees of freedom, F ratios, and significance test for the effects of vegetation type, vegetation removal, microbial addition, and interactions between fixed facots on ammonium and nitrate pool size.

	Ammonium Pool			Nitrate Pool		
Between Subjects	**df**	**F**	**p value**	**df**	**F**	**p value**
VegType	18.67	6.04	**0.010**	18.86	7.190	**0.005**
Crust	16.05	0.93	0.350	17.60	0.370	0.550
Herb	17.59	2.20	0.160	17.35	11.920	**0.003**
Veg:Herb	17.59	0.72	0.410	17.35	5.800	**0.030**
Veg:Crust	15.64	1.75	0.210	16.92	0.410	0.670
Veg:Herb:Crust	15.49	4.41	**0.050**	16.34	0.220	0.640

Figure 2. N cycling rates measured in this experiment including mineralization (a) and consumption (b) rates between sites at all levels of disturbance including the Control (no grass removal or microbial addition treatments), grass removal, microbial addition, and a treatment combination. Values are means ±1 SE (pasture: n = 3-5, disturbed n=4-5, native control: n =2-4). The grass removal treatment was not applied to native scrub sites, only microbial addition.

a.



b.

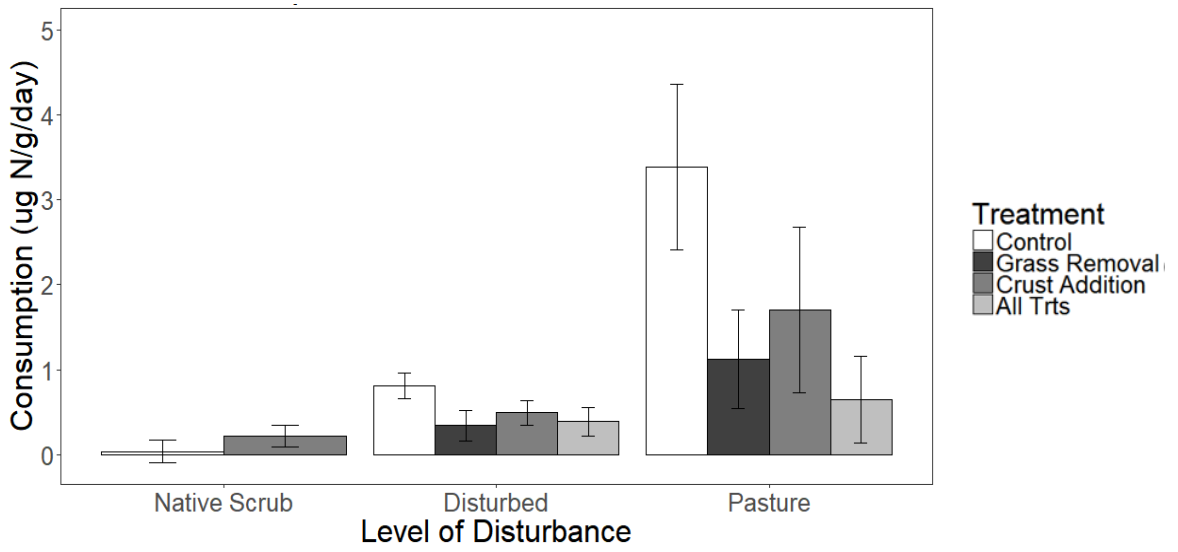
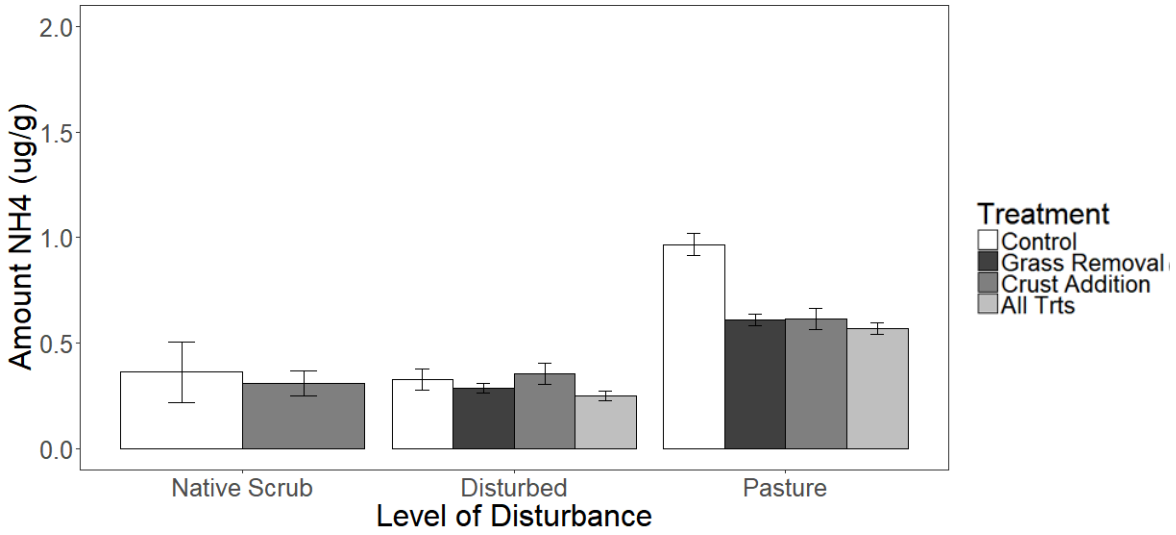


Figure 3. Pool sizes as measured by colorimetric assays. Both the ammonium pool (a) and nitrate pool (b) sizes between sites are shown for all levels of disturbance including the Control (no grass removal or microbial addition treatments), grass removal, microbial addition, and a treatment combination. Values are means ±1 SE (pasture: n = 3-5, disturbed n=4-5, native control: n =2-4). The grass removal treatment was not applied to native scrub sites, only microbial addition.

a.



b.